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Long-term health effects of repeated exposure to multiple vaccines☆

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Abstract

The health of 155 former workers in a US military research program who had received multiple vaccines and 265 matched community controls was assessed. The study population was mostly male (83%) and elderly (median age, 69 years). Multiply immunized (MIP) subjects received vaccines and/or skin tests (median = 154) over a median of 17.3 years; interval from start of immunizations to survey completion was 15–55 years (mean = 43.1 years). MIP subjects characterized themselves as slightly less healthy than controls ($P = 0.057$). Fatigue (but no other symptom) was reported more frequently in the MIP group ($P = 0.011$), but was not associated with number of injections, number of vaccines, or time in program. No differences between MIP and control groups were seen for numerous self-reported medical conditions. Several statistically significant abnormalities were seen in clinical laboratory tests among MIP subjects, but none appeared to be clinically significant. A significant difference in frequency of monoclonal spikes and/or paraprotein peaks between MIP (12.5%) and control (4.5%) groups (RR = 2.7, $P < 0.003$) was observed; no associations with lifestyle, vaccine exposure, or medical conditions were found.
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1. Introduction

The control of communicable diseases through immunization ranks among the most significant of public health achievements. As with any medical intervention, however, the risk–benefit ratio of immunization must be factored into decisions to use vaccines in susceptible individuals. Current regulatory guidelines call for rigorous assessment of new vaccines in animals and in closely controlled human trials to determine adverse reaction profiles before their widespread introduction and licensure. Similarly, existing vaccines are frequently under study to catalogue adverse health outcomes associated with their administration in the general population as well as in specific groups. In most cases, studies of vaccine reactogenicity focus on the consequences of receipt of a single product, and, for reasons of practicality, on the short- and intermediate-term impact on health.

Demonstrating causality between adverse health outcomes and receipt of vaccines can be difficult, particularly for rare events [1]. A variety of surveillance tools (e.g., the Vaccine Adverse Event Reporting System [2]), formal epidemiological studies, and anecdotal reports contribute to understanding potential associations. Recently, anthrax vaccine has been a target of intense study in this regard [3].

The long-term effects, if any, of repetitive vaccination is an area of interest to the scientific and lay communities. The impact of repeated exposure to allergen extracts has been

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14. ABSTRACT

The health of 155 former workers in a US military research program who had received multiple vaccines and 265 matched community controls was assessed. The study population was mostly male (83%) and elderly (median age, 69 years). Multiply immunized (MIP) subjects received vaccines and/or skin tests (median = 154) over a median of 17.3 years; interval from start of immunizations to survey completion was 15-55 years (mean = 43.1 years). MIP subjects characterized themselves as slightly less healthy than controls (P = 0.057). Fatigue (but no other symptom) was reported more frequently in the MIP group (P = 0.011), but was not associated with number of injections, number of vaccines, or time in program. No differences between MIP and control groups were seen for numerous self-reported medical conditions. Several statistically significant abnormalities were seen in clinical laboratory tests among MIP subjects, but none appeared to be clinically significant. A significant difference in frequency of monoclonal spikes and/or paraprotein peaks between MIP (12.5%) and control (4.5%) groups (RR = 2.7, P < 0.003) was observed; no associations with lifestyle, vaccine exposure, or medical conditions were found.

15. SUBJECT TERMS

Whitecoat Project, health status, volunteers, human, vaccines

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examined, and to date, little in the way of long-term adverse health consequences have been documented [4,5]. Similar studies to determine the impact of repeated vaccination against bacterial, viral, or fungal pathogens have been infrequently performed.

Between 1943 and 1969, the United States government was engaged in a program to evaluate potential biological weapons and countermeasures at Fort Detrick, Maryland. Workers in this program were offered a variety of investigational and licensed vaccines against the pathogens under study as adjuncts to environmental (i.e., physical barrier) protection. Three reports were published in 1958, 1963, and 1974 on 99 males who received multiple vaccinations in conjunction with the occupational health program at Fort Detrick [6–8]. No disease or clinical symptom complex could be related to multiple immunization in any of these studies.

The present study was conceived as a follow-up to the previously published experience in the Fort Detrick employee population. The long-term health impact of repeated vaccination was assessed through an analysis of subjective (self-reported) morbidity and laboratory abnormalities in a group of multiply immunized (MIP) individuals who had participated in the Fort Detrick Special Immunization/Special Procedures Programs, and in a group of age-, gender- and race-matched community controls.

2. Materials and methods

2.1. Study design

This was a controlled, unblinded, retrospective cohort study to assess the long-term health effects of repeated inoculation with multiple vaccines and other biologics. Using an unbalanced study design, three age-, race-, and gender-matched controls were sought for comparison with each individual who had received multiple vaccines. Repeated immunization was defined as receipt of the same vaccine or skin test more than three times within 1 year after age 18. An individual was considered to have received multiple immunizations or vaccinations and evaluated for inclusion in the MIP cohort if he or she had five or more different vaccines or skin tests within 1 year after age 18. Included were those individuals who received a single dose of a particular vaccine or skin test antigen (e.g., tularemia) but who also received multiple doses of other antigens (e.g., five doses of plague vaccine). A study questionnaire was developed to obtain demographic, general health, lifestyle, and disease history from all study volunteers. Blood and urine specimens were obtained from all volunteers, and were subjected to a pre-determined battery of laboratory tests. The questionnaire and study protocol were reviewed and approved by the institutional review boards of the US Army Medical Research Institute of Infectious Diseases (Fort Detrick, Maryland) and the Human Subjects Research Review Board of the US Army Surgeon General.

2.2. Study population

Study volunteers were recruited from among the membership of a Fort Detrick alumni group. This group holds biannual social meetings, and the questionnaire was administered to the MIP cohort during one such meeting over the Labor Day holiday in September 1996. The study was explained to potential subjects, and those interested read and signed a written informed consent form. The questionnaire was completed, blood drawn, and urine collected from each volunteer.

A control group of volunteers matched by age (within one decade), gender, and race was recruited from the Frederick, Maryland community, using advertisements in three large newspapers covering in the Baltimore–Washington–Frederick metropolitan areas. Prospective control subjects were assembled in enrollment groups of 5–20 over several months until the desired number had been accrued. At each assembly, the protocol was discussed, individuals were encouraged to ask questions, and those who agreed to participate signed the written informed consent form. The questionnaire was then completed, blood drawn, and urine collected from each volunteer. Upon receipt by study investigators, copies of laboratory results were mailed to study volunteers; subjects were advised to review these results with their primary healthcare providers.

A total of 554 individuals who expressed interest in participating in the study met the entry criteria as MIP or control subjects. Ten had documented cases of illness resulting from exposure to infectious agents/toxins under investigation at Fort Detrick and were excluded from analysis. Eighteen volunteers characterized themselves as other than “white”, and were excluded from analysis due to their small number. An additional 106 redundant controls were omitted from the analysis by random selection within age classes. Ultimately, 155 Caucasian persons who received multiple vaccines, and 265 non-multiply vaccinated controls matched by age, race, and gender, were included in the analysis for this study.

2.3. Immunization histories

Records for receipt of vaccines and skin tests at Fort Detrick were obtained by screening archives of the Special Immunization Program at the US Army Medical Research Institute of Infectious Diseases (Fort Detrick, Maryland). A hand-written copy of each vaccination and skin test received, together with a study record for each participant in the program, was procured and reviewed. The type of vaccine or skin test received as well as the date of receipt of each product were extracted from these records and entered into an automated database for analysis.

2.4. Statistical analysis

Initial demographic analysis compared rates between groups for comparability using Fisher exact tests, and mean

differences using *t*-tests. The groups were discovered to differ in age and sex structure. A decision was made to balance the groups for age by randomly eliminating redundant controls from age classes where the controls were over-represented. This was done using a random-number generator with the goal of achieving a 3:1 ratio between groups across age classes. No study subjects were eliminated. The resulting sample of 265 controls did not differ in sex distribution from the study group ($P = 0.179$), but a small residual age effect was still present ($P = 0.003$). It was decided not to attempt further balancing of the groups as the mean age difference was now quite small (68.7 years in controls versus 70.5 years in the study group). This cohort of 420 subjects was used in all further analyses.

Rate differences between groups were compared by Fisher exact tests, which were adjusted for multiple comparisons using a re-sampling bootstrap approach (SAS MULTTEST) for sets of related variables (symptoms, diseases, atypical laboratory values, etc.). To examine the possibility that confounder variables could explain the group difference, further analysis was done using forward logistic regression analysis on demographic variables of age at interview, sex, cigarette packs-per-day, duration of cigarette smoking, years of education, and reported alcohol use. To examine the effect of exposure variability in the study group by itself, forward logistic regression analysis of specific outcome variables within the study group alone on total antigen loads and durations of exposure was used (years exposed in the MIP, years elapsed since starting in the MIP, total number of doses of all antigens, total volume of all antigens, total number of different antigens, total number of anthrax vaccine doses, total volume of anthrax vaccine, and years of exposure to anthrax vaccine). Attempts were made to assess the effect of individual antigens on outcome variables, but the sample sizes were insufficient for conclusive results. All analyses used SAS Version 8.2 (SAS Institute, Cary, NC).

The statistical power of this study to detect rate differences between groups was assessed using the "Power and Precision" software program [9]. With the sample sizes of 265 controls and 155 study subjects, when testing at the 5% level of significance (one-tailed), a minimum two-fold difference can be detected with at least 80% probability between rates of health-related outcomes over a range of background health condition rates in the controls. In all cases of statistical significance ($P < 0.050$), causality could not be implied by the demonstration of an association between group membership and an outcome variable without further medical interpretation.

3. Results

3.1. Study population characteristics

The majority of study participants were male (83.1%) and elderly (mean age, 69.4 years). Most (67%) had not served in the military. Subjects in the MIP group were slightly older than controls (Table 1). Most individuals in the study (78.1%) had completed high school, and 46.9% had completed college or graduate school; those in the MIP cohort were somewhat better educated than controls (Table 1). More individuals in the MIP group reported being retired from employment than did controls, but most individuals in both groups indicated that they engaged in regular exercise each week (Table 1).

Tobacco use was common among volunteers in both groups (60.9% of study participants reported a history of having smoked cigarettes, 30.2% smoked a pipe, 23.4% smoked cigars, and 6.2% used snuff). However, differences between MIP and control subjects with respect to smoking histories were not generally significant (Table 1). A history of alcohol consumption was common in both groups, with those in the MIP cohort reporting somewhat more use than that among controls; the difference between the two groups was not significant, however (Table 1).

Table 1
Population characteristics

	MIP (N = 155)	Control (N = 265)	P-value[a]
Caucasian	100.0%	100.0%	
Male	86.5%	81.1%	0.179
Mean age (range) (years)	70.5(57–89)	68.7(55–94)	0.003
Served in military	11.0%	46.4%	< 0.001
College degree or higher	57.4%	40.8%	0.001
Current employment status			
Retired	71.6%	63.0%	0.040
Employed full-time	5.2%	6.8%	
Employed part-time	23.2%	26.4%	
Not working/disabled	0.0%	3.8%	
Current exercise level			
None	20.0%	23.5%	0.820
More than 5×/week	17.4%	17.0%	
Up to 5×/week	60.0%	57.6%	
Disabled	2.6%	1.9%	
Tobacco history			
Ever smoked cigarettes	64.5%	58.7%	0.255
#Packs/day (mean)	1.2	1.3	0.173
#Years smoked (mean)	21.7	25.5	0.068
Quit	90.9%	86.8%	0.420
Years since quitting (mean)	25.6	22.6	0.087
Ever smoked pipe	36.4%	26.6%	0.046
Ever smoked cigars	25.7%	22.1%	0.470
Alcohol use			
Ever drink	30.3%	22.6%	0.083
Hepatitis markers			
Hepatitis A antibody	51.0%	53.0%	0.714
Anti-hepatitis B core antibody	5.0%	8.0%	0.945
Hepatits B surface antigen	0.0%	0.0%	NT
Anti-hepatitis C antibody	0.0%	2.0%	1.000
HLA-B27	9.8%	10.6%	0.868

NT, not tested.

[a] Tests are two-tailed except hepatitis markers which test only for elevation in study group percentage compared to controls.

The distribution of markers for hepatitis A, hepatitis B, hepatitis C, and HLA B27 was similar between groups (Table 1), supporting comparability of the two study populations.

3.2. Vaccine/skin test exposures

Subjects in the MIP cohort participated in the MIP for a median of 17.3 years (range, 1.2–44.0 years). During this period, individuals typically received a large number of vaccinations and/or skin tests (median number, 154; range, 24–354); the median and mean volumes of material inoculated were 57.2 ml/individual and 58.7 ml/individual, respectively (range, 8.8–135.4 ml). Females tended to be somewhat less "exposed" than males (median, 16.0 years in MIP; median, 36.4 ml total antigenic volume received; median, 68.0 total number of MIP injections for females versus median 19.1 years in MIP; median, 60.0 ml total antigenic volume received; and median, 126.5 total number of MIP injections received for males). However, the number of females available for study was small ($n = 21$). The time between the start of vaccinations and/or skin tests and participation by volunteers in the current study ranged from 15 to 55 years (median = 43.1 years, mean = 43.0 years).

A total of 38 different vaccines and/or skin tests ("antigens") were administered by the Special Immunizations Program to the MIP cohort during the course of their employment at Fort Detrick (Table 2). Most of these products were unlicensed, and were administered under approved investigational new drug (IND) protocols. The most common antigen exposures (in the form of vaccines and/or skin tests) were tularemia (96.8% of the cohort), Venezuelan equine encephalitis virus (93.5%), anthrax (91.6%), *Brucella* (91.0%), plague (89.0%), tuberculin (88.4%), *Vaccinia* virus

Table 2
Vaccine and skin test exposures among MIP subjects

Vaccine	Total doses administered (*n*)	#Subjects receiving product (*n*)	#Doses/subject		Antigenic volume/subject (ml)	
			Mean	Range	Mean	Range
Tularemia	4376	150	29.2	1–78	5.3	0.1–17.4
Anthrax	3241	142	22.8	3–50	6.7	0.7–17.3
Plague	2510	138	18.2	1–44	6.4	1.0–14.8
Botulinum toxoid (ABCDE)	1709	136	12.6	2–36	6.5	1.0–21.8
Venezuelan equine encephalitis (VEE)	1644	145	11.3	1–38	6.3	0.5–16.8
Vaccinia	1161	136	8.5	1–37	0.2	<0.1–1.7
Tuberculin	1074	137	7.8	1–74	0.6	<0.1–5.7
Brucella	905	141	6.4	1–28	0.7	0.1–2.9
Influenza	768	129	6	1–15	5.8	0.5–15.0
Q-fever	756	119	6.4	1–16	4	0.1–9.1
Psittacosis	675	100	6.8	1–26	4.9	0.5–19.1
Rocky mountain spotted fever	636	113	5.6	1–17	4.4	0.5–11.5
Coccidiodomycosis	615	112	5.5	1–22	0.5	0.1–2.2
Rift Valley fever	570	121	4.7	1–23	4.7	1.0–23.0
Eastern equine encephalitis (EEE)	471	65	7.2	1–20	1.5	0.1–4.5
Tetanus toxoid	436	130	3.4	1–13	1.7	0.5–10.0
Yellow fever	358	131	2.7	1–8	1.4	0.5–4.0
Typhus	350	79	4.4	1–14	3.2	0.5–11.5
Western equine encephalitis (WEE)	335	50	6.7	1–20	1.4	0.2–4.9
Histoplasmosis	275	128	2.1	1–9	0.2	0.1–0.9
Polio	265	98	2.7	1–6	2.5	0.5–5.0
EEE/WEE/VEE[a]	253	62	4.1	1–7	2	0.5–3.5
EEE/WEE[a]	236	77	3.1	1–8	1.2	0.1–2.0
RMSF/Q-fever/typhus[a]	159	45	3.5	1–6	1.7	0.3–3.3
Cholera	134	14	9.6	1–31	5.2	0.5–16.0
Blastomycosis	128	32	4	1–10	0.4	0.1–1.0
Typhoid	77	22	3.5	1–13	1.4	0.1–5.3
Japanese encephalitis	44	14	3.1	1–4	2.9	1.0–3.5
Diphtheria	42	14	3	1–5	0.7	0.1–1.3
Rabies	37	8	4.6	3–9	4.4	3.0–8.2
Chikungunya	27	14	1.9	1–3	1	0.5–1.5
Glanders	18	12	1.5	1–2	0.2	0.1–0.2
Hepatitis B	17	4	4.3	3–7	4.1	3.0–6.5
Tickborne encephalitis	11	2	5.5	2–9	2.8	1.0–4.5
EEE/VEE[a]	9	9	1	1–1	0.3	0.1–0.5
Mumps	7	7	1	1–1	0.1	0.1–0.1
Junin	3	3	1	1–1	1	0.9–1.0
Dengue 2	1	1	1	1–1	0.5	0.5–0.5

[a] Polyvalent vaccine/skin test; component antigens may or may not be the same as monovalent products.

(87.7%), botulinum toxoid (87.7%), yellow fever virus (84.5%), tetanus toxoid (83.9%), influenza virus (83.2%), histoplasmosis (82.6%), Rift Valley fever virus (78.1%), Q fever (76.8%), Rocky Mountain spotted fever (72.9%), and coccidiodomycosis (72.3%).

Many antigens were administered on multiple occasions to MIP volunteers over the course of their employment. The total number of doses of vaccines and skin tests administered to the study cohort was 24,333. The greatest numbers of products administered were tularemia (4376 doses), anthrax (3241 doses), plague (2510 doses), botulinum toxoid (1709 doses), Venezuelan equine encephalitis virus (1644 doses), and *Vaccinia* virus (1161 doses) (Table 2). Because the dosing schedule for each vaccine depended upon a variety of factors (e.g., live versus inactivated, immunogenicity, use of skin test, etc.), the numbers of antigen doses received per individual varied widely (from 1 to 78); tularemia (mean = 29.2 doses/recipient), anthrax (mean = 22.8 doses/recipient), and plague (mean = 18.2 doses/recipient) were the antigens most frequently administered. The median number of different antigens administered per subject was 19 (range, 5–27).

The total volume of vaccines and skin tests received by the MIP cohort was 9104 ml. Anthrax (up to 17.3 ml), botulinum toxoid (up to 21.8 ml), plague (up to 14.8 ml), Venezuelan equine encephalitis virus (up to 16.8 ml), influenza virus (up to 15.0 ml), tularemia (up to 17.4 ml), cholera (up to 16.0 ml), psittacosis (up to 19.1 ml), and Rift Valley fever virus (up to 23.0 ml) represented the greatest antigenic volumes administered to individuals in the study population (Table 2).

3.3. Outcomes

MIP subjects tended to characterize themselves as somewhat less healthy than controls (current state of health= "excellent": 26.0% of MIP group versus 37.4% of controls; "good": 62.3% of MIP versus 53.2% of controls; "fair–poor": 11.7% of MIP versus 9.4% of controls); however, differences did not reach statistical significance ($P = 0.057$, chi-square for trend). Factors associated (using forward-selection logistic regression) with poorer general health in the entire study population included fewer years of education ($P < 0.001$), increased smoking burden (cigarette packs-per-day) ($P < 0.001$), and increased age at interview ($P = 0.003$). In a search for prognostic variables associated with poorer general health among MIP participants by forward-selection logistic regression, fewer years of education ($P < 0.001$), increased age at interview ($P = 0.024$), and increased duration of cigarette smoking ($P = 0.042$) proved significant. MIP-specific exposure variables, including the total number of injections (vaccinations and skin tests), total injection volumes, numbers of different vaccines and skin tests, number and volume of anthrax vaccinations, duration of anthrax vaccine exposure, duration of MIP participation, and interval from MIP enrollment to study enrollment, were not associated.

When study volunteers were questioned about a variety of clinical signs and symptoms, only fatigue was associated more frequently with a higher rate in the MIP group ($P = 0.011$) (Fig. 1). This group association remained after pooling severe and moderate categories and adjusting for multiple comparisons ($P = 0.037$, RR =1.48, 95% CI = 1.11–1.97). After adjusting for age, sex, cigarette use, alcohol use, and education level, fatigue remained associated with MIP group membership. No association was found between fatigue and antigen exposure variables (e.g., total number and volume of vaccines and skin tests received, or specific antigens), nor was it associated with monoclonal gammopathy, laboratory abnormalities, or any self-reported disease condition except arthritis (40.1% versus 24.9%, $P = 0.008$, adjusted for multiple comparisons). No differences were observed for a wide variety of self-reported medical conditions between MIP subjects and controls (Table 3). None of these variables predicted MIP or control group membership in the general health analysis.

A comparison of clinical chemistry and hematology panels obtained from MIP and control subjects yielded statistically significant differences between study groups in out-of-normal-range values for several tests: serum calcium (11.7% of MIP subjects had values above the normal reference range compared with 0 controls [one-tailed $P < 0.001$ adjusted for multiple comparisons]), serum bicarbonate (7.1% of MIP subjects versus 1.5% of controls had values below the normal reference range [adjusted $P = 0.044$]), percent thyroxine uptake (33.1% of MIP subjects had values above the normal reference range versus 12.5% of controls [adjusted $P < 0.001$]), serum alpha-2 globulin percent (6.5% of MIP subjects versus 0% of controls were below the normal reference range [adjusted $P = 0.001$]), and serum IgG4 (4.6% of MIP subjects versus 0% of controls had levels below the normal reference range [adjusted $P = 0.005$]) (Table 4 and Fig. 2). A significantly higher proportion of MIP subjects had abnormally high serum glucose values, but no effort was made to control fasting status prior to phlebotomy (data not shown).

A greater proportion of MIP subjects than controls displayed red blood cell mean corpuscular hemoglobin (MCH) (31.2% versus 10.6%, adjusted $P < 0.001$) and mean corpuscular volume (MCV) (22.7% versus 7.6%, adjusted $P = 0.001$) values above the normal reference range; no differences in other red blood cell indices were observed, however (Fig. 3). The proportion of individuals with mean platelet volumes below the normal reference range was significantly lower among MIP volunteers than among controls (19.0% versus 5.3%, adjusted $P < 0.001$), but no differences in the proportion of individuals with abnormal platelet numbers were observed between the groups (Fig. 3).

Cell markers consistent with a diagnosis of chronic lymphocytic leukemia were seen in five individuals (two in the MIP group and three controls). One of these individuals (in the MIP group) reported a history of leukemia. In another case (also in the MIP group), a monoclonal spike was identified by serum protein electrophoresis. Cell marker studies were not systematically performed for this study, however. Assays were performed in only 14 individuals; indications

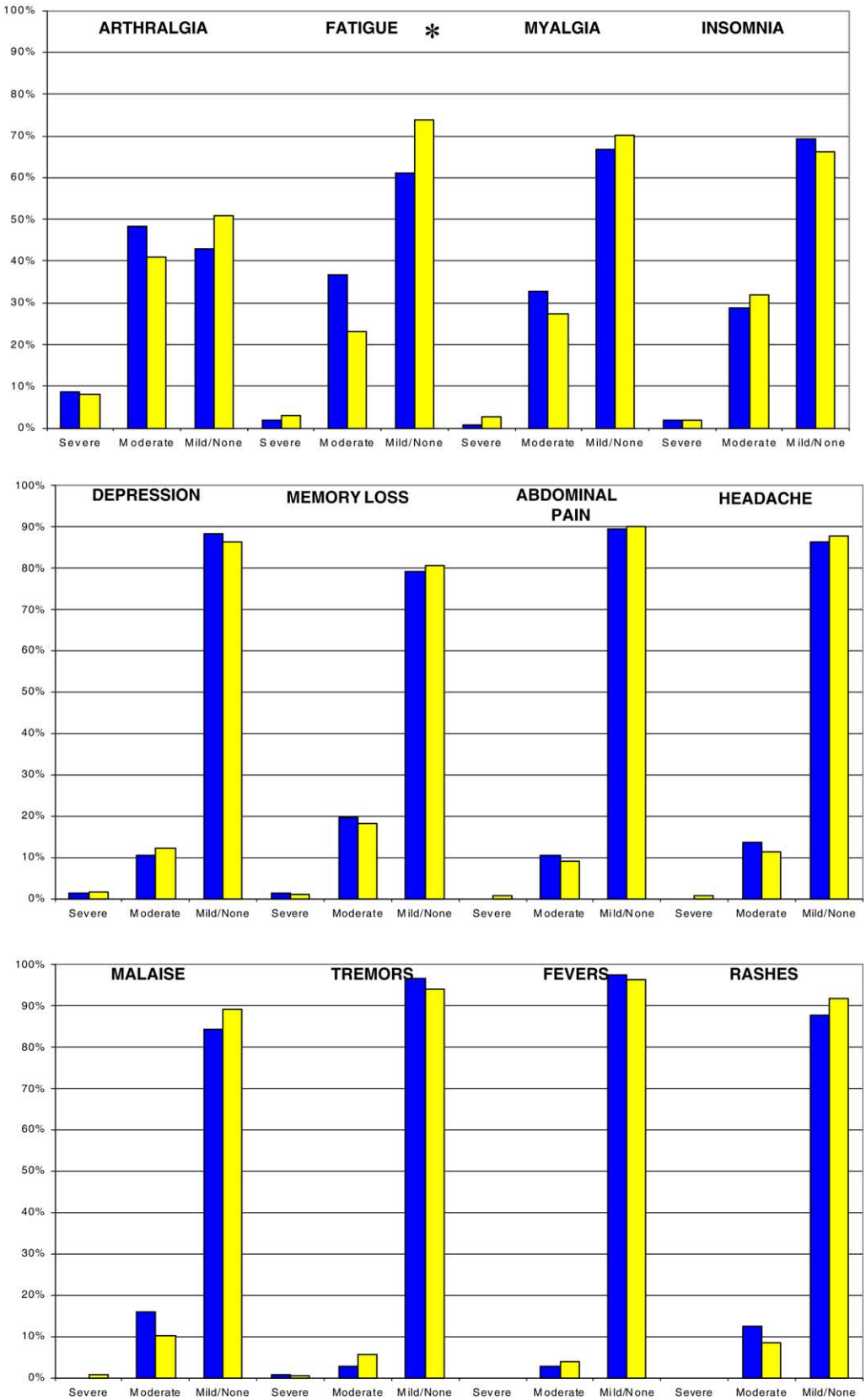

Fig. 1. Distribution of self-reported symptoms among MIP (dark-colored bars) and control (light-colored bars) subjects by severity (severe, moderate, or mild/none). Asterisk denotes symptom for which a statistically significant difference between MIP and control subjects was observed (see text for details).

Table 3
Self-reported diseases and conditions

Condition	MIP (*N*)	Control (*N*)	MIP (%)	Control (%)	*P*-value[a]
Arthritis	58	100	37.4	37.7	1.000
Hypertension	56	75	36.1	28.3	0.515
Pneumonia	26	43	16.8	16.2	1.000
Cancer	24	33	15.5	12.5	0.967
Hay fever	22	32	14.2	12.1	0.998
Ulcers	14	27	9	10.2	1.000
Diabetes	14	18	9	6.8	0.975
Asthma	11	23	7.1	8.7	1.000
Thyroid disease	9	14	5.8	5.3	1.000
Rheumatoid arthritis	9	9	5.8	3.4	0.930
Eczyma	9	7	5.8	2.6	0.671
Anemia	8	12	5.2	4.5	1.000
Kidney disease	6	6	3.9	2.3	0.975
Frequent colds	5	14	3.2	5.3	1.000
Leukopenia	5	5	3.2	1.9	0.992
Atopic dermatitis	4	1	2.6	0.4	0.563
Platelet problems	4	1	2.6	0.4	0.563
Iron deficiency anemia	3	4	1.9	1.5	1.000
Parkinson's disease	2	1	1.3	0.4	0.996
Pneumonitis	2	1	1.3	0.4	0.996
Serum sickness	2	0	1.3	0	0.862
Vasculitis	2	0	1.3	0	0.862
Vitamin B12 deficiency	1	6	0.6	2.3	1.000
Lupus	1	2	0.6	0.8	1.000
Erythema nodosum	1	0	0.6	0	0.999
Immune complex disease	1	0	0.6	0	0.999
Leukemia	1	0	0.6	0	0.999
Neuritis	0	3	0	1.1	1.000
Temporal arteritis	0	2	0	0.8	1.000
Amyloidosis	0	1	0	0.4	1.000
Guillain–Barre syndrome	0	1	0	0.4	1.000
Anemia of chronic disease	0	0	0	0	1.000
Aplastic anemia	0	0	0	0	1.000
Glomerulonephritis	0	0	0	0	1.000
Goodpasture's syndrome	0	0	0	0	1.000
Hemolytic anemia	0	0	0	0	1.000
Hodgkins disease	0	0	0	0	1.000
Multiple myeloma	0	0	0	0	1.000
Multiple sclerosis	0	0	0	0	1.000
Reiter's syndrome	0	0	0	0	1.000
Sarcoidosis	0	0	0	0	1.000
Sjogren's syndrome	0	0	0	0	1.000
Uveitis	0	0	0	0	1.000
Wegeners granulomatosis	0	0	0	0	1.000
Total[b]	300	441	4.4	3.8	

[a] Fisher exact test (one-tailed) for increasing risk in the MIP group compared to controls, adjusted for multiple comparisons.
[b] Total reported conditions and percent of total answered questions.

were based upon findings from routine hematological analyses and/or clinical history (i.e., self-reported leukemia).

Results of a variety of immunologic/rheumatologic assays yielded no differences between MIP subjects and controls. Specifically, there was no indication that MIP subjects were more apt to possess autoimmune antibodies than were control individuals (Table 5).

Among MIP subjects, regression analysis of health outcomes (laboratory abnormalities and self-reported diseases/conditions) using forward-selection logistic regression on MIP exposure variables yielded associations between rheumatoid arthritis and duration of cigarette smoking ($P < 0.001$), cancer and age ($P = 0.002$), and diabetes and smoking burden (cigarette packs-per-day) ($P = 0.029$).

3.4. Monoclonal gammopathies

Serum electrophoresis and immunofixation electrophoresis studies yielded abnormal findings for 81 individuals. Of these, 50 were interpreted as non-specific (e.g., patterns consistent with acute or chronic inflammation). There was no difference in distribution of non-specific abnormalities between MIP and control groups (17/153 [11.1%] in MIP subjects versus 33/265 [12.5%] in controls; $P = 0.711$, unadjusted). However, monoclonal spikes and/or paraprotein (abnormal immunoglobulin or immunoglobulin light chain) peaks were observed in 31 subjects 19 (12.4%) in the MIP group and 12 (4.5%) in the control group (Table 6). This difference in monoclonal protein prevalence between groups was significant (RR =2.7 [95% CI = 1.4–5.5]; $P = 0.003$ [adjusted for multiple comparisons, $P = 0.034$]).

Nine of the 31 monoclonal proteins were identified as IgM by immunofixation electrophoresis (four in the MIP group and five in controls), 13 were IgG (eight in the MIP group and five in controls), five were IgA (four in the MIP group and one in the control group), while four monoclonal peaks could not be further characterized with regard to immunoglobulin class (three in the MIP group and one among controls). Kappa light chains were found in 16 individuals (12 in the MIP group and four among controls), lambda light chains were found in 14 individuals (six in the MIP group and eight among controls), while in one MIP subject, light chains could not be adequately characterized.

A search for demographic, lifestyle, exposure (including total number, total volume, or type of vaccine antigen), or other disease associations yielded little in the way of positive findings. Those in the MIP group who had serum monoclonal proteins identified tended to be slightly older on average than controls with monoclonal proteins (74.4 years versus 67.2 years, $P < 0.001$). When the group difference (MIP versus control) was adjusted for age in a logistic regression model, however, the effect of group remained significant ($P = 0.011$). Thus, the age differential appeared not to be the cause of observed differences between groups. No associations between the presence of monoclonal proteins and self-reported diseases or conditions were seen; specifically, those with monoclonal proteins reported no cases of amyloidosis or multiple myeloma.

Serum calcium values exceeding the upper range of normal were seen in three individuals with monoclonal gammopathy. All increases were modest, however (10.5–10.8 mg/dl; laboratory upper range of normal = 9.9 mg/dl), and although the frequency of hypercalcemia in this group (9.7%) was higher than in those without serum paraproteins (3.9%), the difference was not statistically significant. Similarly, no significant differences were observed

Table 4
Protein and immunoglobulin measures

Test	MIP			Control			*P*-value[a]	
	n	Out of reference range, low (%)	Out of reference range, high (%)	*n*	Out of reference range, low (%)	Out of reference range, high (%)	Out of range, low	Out of range, high
Albumin (%)	153	10.5	20.3	265	10.6	12.5	0.997	0.238
Albumin (g/dl)	153	3.3	1.3	265	2.6	0.0	0.993	0.851
Total protein (g/dl)	153	0.7	0.7	265	0.8	1.5	1.000	1.000
Alpha-1 globulin (%)	153	1.3	2.0	265	0.8	1.5	0.994	1.000
Alpha-1 globulin (g/dl)	153	0.0	1.3	265	0.0	1.1	1.000	1.000
Alpha-2 globulin (%)	153	6.5	1.3	265	0.0	1.9	0.001	1.000
Alpha-2 globulin (g/dl)	153	0.7	0.0	265	0.0	1.5	0.970	1.000
Beta globulin (%)	153	0.0	2.0	265	0.0	1.9	1.000	1.000
Beta globulin (g/dl)	153	0.0	2.6	265	0.0	2.6	1.000	1.000
Gamma globulin (%)	153	2.0	1.3	265	1.1	2.3	0.981	1.000
Gamma globulin (g/dl)	153	1.3	3.9	265	0.4	6.4	0.927	1.000
C-4 (mg/dl)	153	0.0	31.4	265	0.0	22.3	1.000	NT[b]
Copper (ug/dl)	153	3.9	1.3	265	1.9	3.4	0.725	1.000
C-reactive protein (mg/dl)	153	0.0	5.2	265	0.0	10.9	NT	1.000
IgM (mg/dl)	153	4.6	9.8	265	5.3	5.3	1.000	0.553
IgG (mg/dl)	153	1.3	9.2	265	1.9	12.1	1.000	1.000
IgA (mg/dl)	153	3.3	9.2	265	2.3	10.9	0.975	1.000
Total IgG (mg/dl)	150	0.7	3.3	261	0.0	10.3	0.959	1.000
IgG1 (mg/dl)	152	0.7	2.0	265	0.8	4.2	1.000	1.000
IgG2 (mg/dl)	152	0.0	13.2	264	0.0	14.8	1.000	1.000
IgG3 (mg/dl)	152	0.7	1.3	264	1.1	7.2	1.000	1.000
IgG4 (mg/dl)	152	4.6	0.0	261	0.0	0.0	0.005	1.000

[a] One-tailed upper tested for MIP > control group, adjusted for multiple comparisons.
[b] Not tested.

Table 5
Rheumatological assays

Test	MIP		Control		*P*-value[a]
	n	%	*n*	%	
Anti-thyroglobulin antibody (1:20 or greater)	3	2.00	6	2.30	1.000
Anti-thryoid microsomal antibody (1:100 or greater)	7	4.60	16	6.00	1.000
Hep-2 ANA titer (1:40 or greater)	80	52.30	147	55.50	1.000
ANA fluorescence pattern					
None detected	73	47.70	118	44.50	
Centromere	0	0.00	2	0.80	0.555[b]
Nucleolar	1	0.60	2	0.80	
Speckled	79	51.60	139	52.40	
Speckled nucleolar	0	0.00	4	1.50	
Mouse kidney/stomach ANA titer (1:40 or greater)	19	12.40	26	9.80	0.975
Fluorescence pattern					
None detected	134	87.60	239	90.20	0.616[b]
Nucleolar	1	0.60	1	0.40	
Speckled	18	11.80	25	9.40	
Quantitative RF (Iu/ml)	17	11.10	27	10.20	1.000

[a] One-tailed upper tested for MIP > control group, adjusted for multiple comparisons.
[b] Fisher exact test for trend (unadjusted for multiple comparisons).

in frequencies of elevated blood urea nitrogen (12.9% versus 14.5%) or creatinine (3.2% versus 5.4%).

4. Discussion

In this cohort of 155 former laboratory workers, we were unable to associate any disease or medical condition with intensive vaccination with multiple antigens or any single antigen. Our findings are consistent with previous comprehensive health assessments performed in multiply-vaccinated laboratory workers conducted over 25 years [6–8]; this study extends the follow-up interval for individuals receiving multiple vaccines by another 20–25 years (to 1996). In the earlier studies, no disease or unusual clinical symptom complex related to multiple immunization

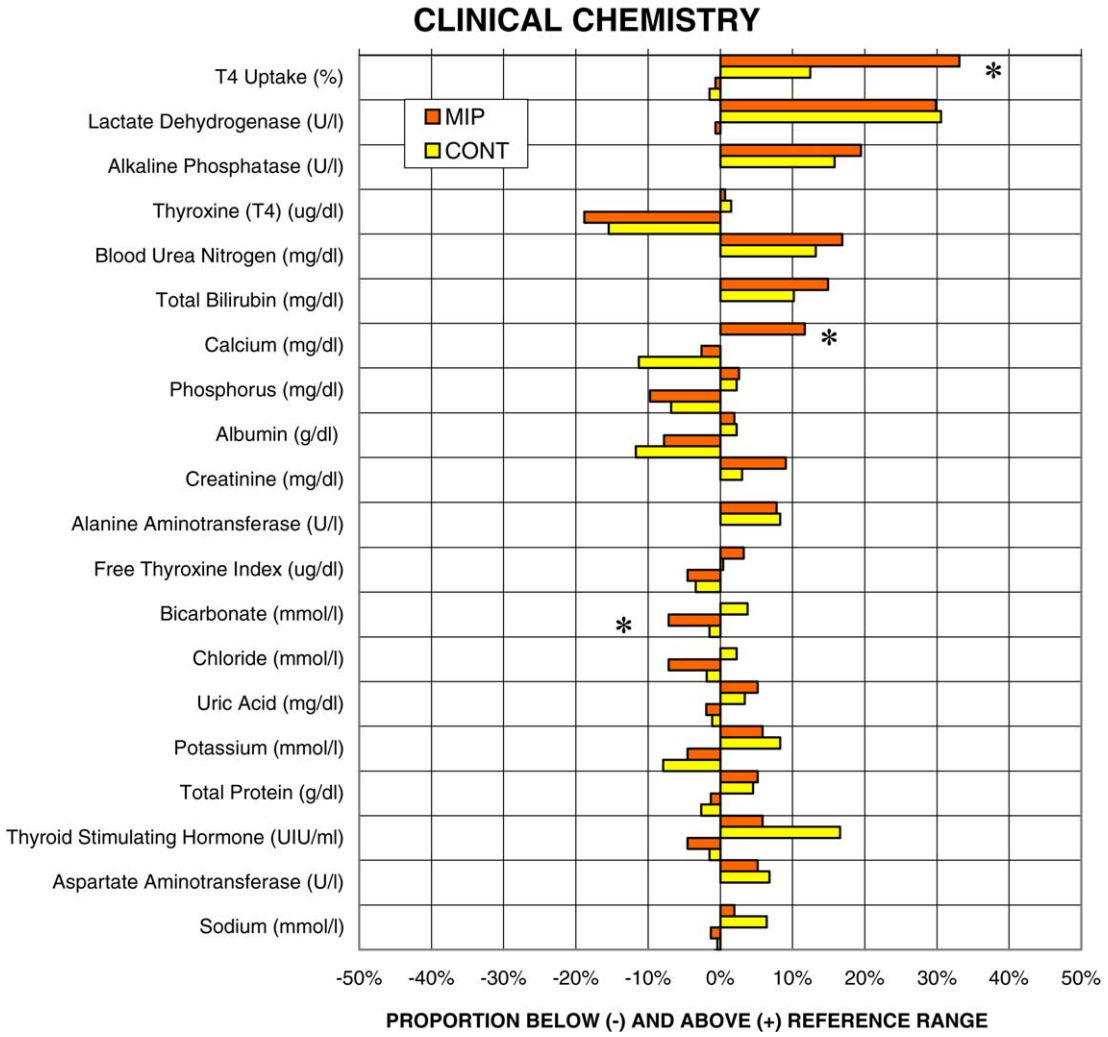

Fig. 2. Comparison of abnormal serum chemistry values for MIP (dark-colored bars) and control (light-colored bars) subjects. The proportions of each group with values above and below the laboratory reference range for each test are displayed as positive and negative, respectively. Asterisk denotes test for which a statistically significant difference between MIP and control subjects was seen (one-tailed *P*-value adjusted for multiple comparisons).

was identified. A small number of laboratory abnormalities were observed in some individuals, including elevated white blood cell counts, abnormal blood protein electrophoretic patterns, elevated erythrocyte sedimentation rate, elevated levels of selected blood chemistry studies (specifically, serum hexosamine and copper levels), and depressed serum iron values. None of these findings was considered clinically significant, and none supported a diagnosis of disease or illness. The investigators concluded, "These data and the accompanying evaluation of an intensively immunized population provide evidence that no obvious adverse effects resulted from repeated immunization." [8].

Our study was not population-based and is, therefore, subject to biases inherent in volunteer cohort analyses. The number of individuals participating in the previous studies who also participated in the current project is unknown. No effort was made to access mortality records, and it is therefore possible that serious disease associations with intensive vaccination were missed during the interval between the previous assessment of the group and the present. In the course of our laboratory evaluations, five previously undetected cases of chronic lymphocytic leukemia were identified by flow cytometry (studies prompted by abnormalities found through routine hematological studies); in one of these cases (subject study 0A47, Table 6), a monoclonal spike was also detected by serum protein electrophoresis. Although the numbers are quite small, statistical analysis showed no difference in distribution of leukemia diagnoses between MIP and control groups.

Multiply vaccinated study subjects characterized themselves as somewhat less healthy than age- and gender-matched controls, but the differences between the two groups were of only borderline significance. Importantly, vaccination-related variables (number of injections, injection volumes, numbers of different vaccines and skin tests, number of anthrax vaccinations, volume of anthrax vaccinations, duration of anthrax vaccine exposure, duration of MIP participation, and interval from MIP enrollment to study

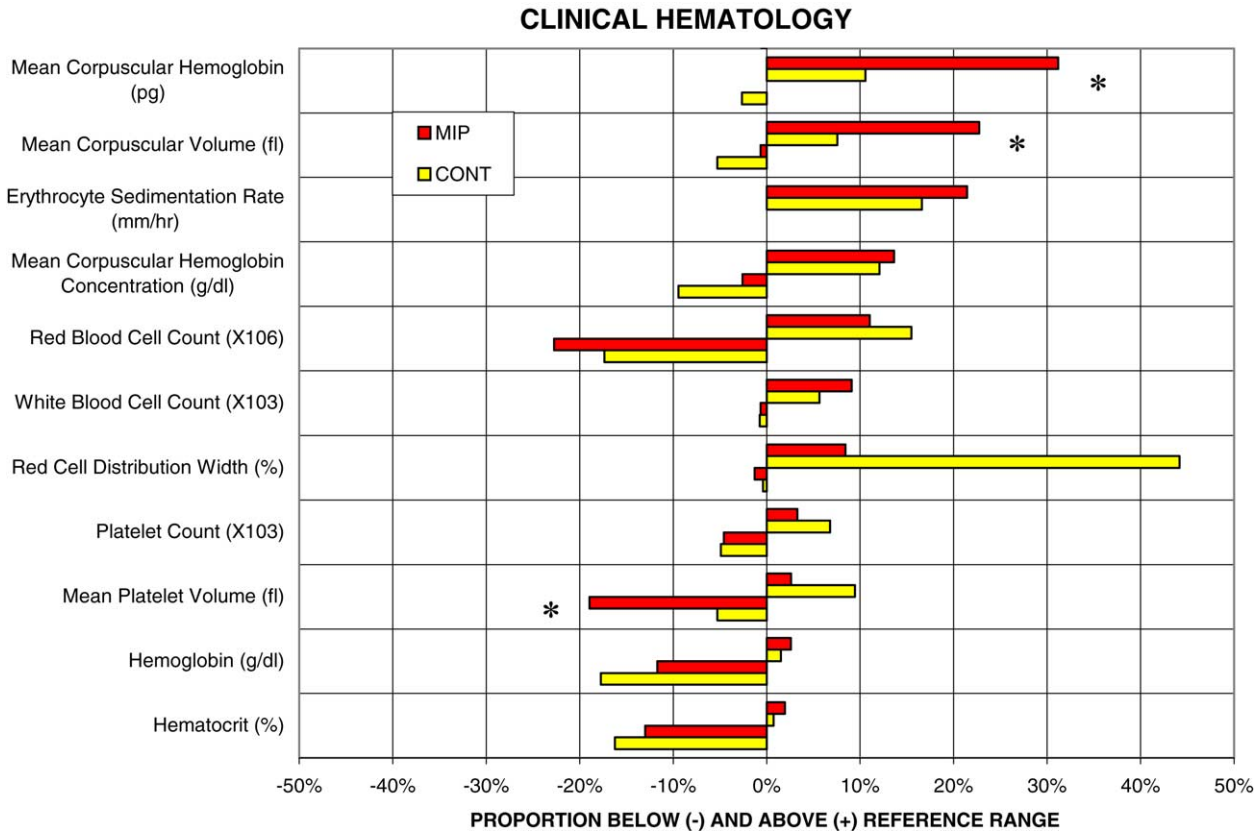

Fig. 3. Comparison of abnormal clinical hematology values for MIP (dark-colored bars) and control (light-colored bars) subjects. The proportions of each group with values above and below the laboratory reference range for each test are displayed as positive and negative, respectively. Asterisk denotes test for which a statistically significant difference between MIP and control subjects was seen (one-tailed *P*-value adjusted for multiple comparisons).

enrollment) were not associated with overall current health status.

From among a variety of self-reported symptoms, only fatigue was statistically associated with receipt of multiple vaccines. Multivariate analysis failed to identify further linkages to number of shots, number of antigens or time in the multiple immunizations program. The study detected statistically significant deviations of several laboratory values from the normal ranges. However, none of these was deemed to be of clinical significance, and none was associated with specific clinical conditions or symptoms.

The most important finding of this study was the increase in prevalence of monoclonal proteins noted among MIP subjects compared with controls. The significance and implications of this finding are unclear, but deserve further investigation. Monoclonal gammopathies are characterized by the proliferation of a single clone of plasma cells, resulting in the production of a homogeneous monoclonal protein detectable by electrophoresis and immunofixation [10]. Abnormalities described as "merging of the α_2 and β globulins" were noted on serum protein electrophoreses performed on members of this population previously. Notably, however, serum paraproteins were not observed [6–8]. In striking contrast, monoclonal proteins were identified in the serum of 31 individuals in our cohort (7.4%, overall). Unfortunately, the nature of the present study precluded further elaboration of the nature of these abnormalities. None of our MIP or control subjects with serum paraproteins reported a diagnosis of plasma cell dyscrasia, and none displayed excessively high serum calcium, urea nitrogen, or creatinine values suggesting underlying disease. While results of more definitive evaluation of the monoclonal gammopathies by study subjects' personal physicians were unavailable to us, it is likely that most of these individuals had monoclonal gammopathy of undetermined significance (MGUS).

MGUS is a condition characterized by the presence of a monoclonal protein in persons without evidence of multiple myeloma, Waldenstrom's macroglobulinemia, amyloidosis, or a related plasma cell disorder [10]. The prevalence of MGUS is around 3% in persons older than 70 years in the US [11,12], but increases significantly with advancing age [13,14]. The observed prevalence of monoclonal serum protein gammopathy observed among controls in our study (4.5%) is consistent with these population-based rates.

MGUS is defined by the presence of serum monoclonal protein at a concentration less than 3 g/dL; no monoclonal protein or only moderate amounts of monoclonal light chains in the urine; the absence of lytic bone lesions, anemia, hypercalcemia, and renal insufficiency related to the monoclonal protein; and (if determination is made) plasma cells compris-

Table 6
Monoclonal paraproteins

Volunteer	Finding	Sub-class	Chain
Study-8V97	Monoclonal spike	IgM	lambda
Study-6B99	Monoclonal spike	IgG	lambda
Study-9H18	Monoclonal spike	Unk[a]	Unk[a]
Study-5N01	Monoclonal spike	IgM	kappa
Study-8P02	Monoclonal spike	IgM	kappa
Study-4S19	Paraprotein	Unk	kappa
Study-7E15	Monoclonal spike	IgG	kappa
Study-1M64	Monoclonal spike	IgG	kappa
Study-5W67	Monoclonal spike	IgA	lambda
Study-8R54	Paraprotein	IgG	kappa
Study-1U44	Paraprotein	IgA	kappa
Study-5S26	Monoclonal spike	IgG	lambda
Study-5D45	Paraprotein	IgA	kappa
Study-2Q41	Paraprotein	IgG	lambda
Study-3C28	Monoclonal spike	IgA	lambda
Study-7Y83	Paraprotein	IgG	kappa
Study-7K87	Paraprotein	Unk[a]	kappa
Study-8L76	Monoclonal spike	IgM	kappa
Study-0A47	Monoclonal spike	IgG	kappa
Control-3V34	Paraprotein	IgM	kappa
Control-0N06	Paraprotein	IgA	lambda
Control-1G06	Monoclonal spike	IgG	kappa
Control-3J71	Monoclonal spike	IgM	kappa
Control-4X66	Paraprotein	Unk[a]	lambda
Control-0Z41	Monoclonal spike	IgG	lambda
Control-0W41	Paraprotein	IgM	lambda
Control-2T41	Monoclonal spike	IgM	kappa
Control-4C44	Monoclonal spike	IgG	lambda
Control-6L20	Monoclonal spike	IgG	lambda
Control-5W15	Monoclonal spike	IgG	lambda
Control-9C83	Monoclonal spike	IgM	lambda

[a] Unk, unknown/indeterminate.

ing 10% or less of cells in the bone marrow [10]. Myeloma cells are generally localized to the bone marrow, are long-lived (based upon label uptake indices), and have significantly lower rates of Ig secretion than normal plasma cells. The observation that monoclonal proteins secreted by these cells may possess homogeneous antigen-binding capabilities [15] is compatible with later findings that somatic mutations in myeloma cell Ig genes occur at a much higher frequency than in other tumor types, in a manner strongly suggestive of prior antigenic selection pressure, but without evidence for continuing hypermutation [16]. Therefore, malignant transformation is currently thought to involve long-lived plasma cells (versus plasmablasts or short-lived plasma cells) after normal differentiation has occurred [17].

The risk of developing multiple myeloma after a diagnosis of MGUS has been determined to be about 1% per year, with concentration of the monoclonal protein (perhaps a surrogate for plasma cell burden) being the most important predictor of progression [18,19]. However, not all patients with MGUS are destined to develop a more serious disease. In a cohort of MGUS patients studied at the Mayo Clinic, approximately 10% of individuals with MGUS followed for 24–38 years did not progress, while in 5%, the monoclonal protein disappeared [10]. The risk of progression to a more malignant outcome for individuals in whom serum monoclonal proteins were found during evaluations for our study is unknown.

The role of commonly observed cytogenetic changes in creation of a MGUS clone and its subsequent malignant transformation are active areas of investigation [10]. More fundamentally, the genesis of Ig heavy chain locus translocations found in the majority of individuals with multiple myeloma [20,21] and half of those with MGUS [22,23], which result in oncogene dysregulation and putative tumor suppressor gene suppression, is undefined [17,24]. Chronic immune stimulation stands as one biologically plausible possibility. The development of amyloidosis in experimental animals after hyperimmunization has been well documented [25–27], but adjuvant effects [28] and host genetics [29] play an as yet undefined role in this process. Plasmacytosis, glomerulonephritis, and vasculitis occur frequently with Aleutian disease in mink, and have been linked to persistent immune stimulation by the causative agent, a parvovirus [30,31]. Reports of plasma cell dyscrasias [32–34] and vasculitis [35] after prolonged administration of allergens to promote allergic hyposensitization suggest that prolonged stimulation of the reticuloendothelial system may promote aberrant immune system behaviors in some humans. Such complications are apparently infrequent, however, as supported by failure to identify similar phenomena among populations of atopic patients receiving long-term hyposensitization therapy in allergy clinics [4,5].

The magnitude (nearly three-fold) of the increased prevalence of serum paraproteinemia among elderly individuals whose immune systems had been repeatedly stimulated through receipt of multiple vaccines was surprising. The implications of this observation for the long-term health of our study subjects are unknown. Follow-up of living members of the monoclonal gammopathy cohort is under consideration, and a larger study drawn from, approximately, 3000 volunteers who have participated in our Institute's Special Immunization Program may elucidate these findings further through review of mortality records and targeted laboratory assessments among living volunteers.

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